

CLAIMS

1. Pair of oligonucleotides, for use as a set in the amplification of a target sequence of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATTT TCTACAGGTG TTAACCTAGT
AGCTGTACCG ACTGGTTATG TTGACACTGA AAATAACACA GAATTCACCA
GAGTTAATGC AAAACCTCCA CCAGGTGACC AGTTTAAACA TCTT,

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC
AGAAGCTTCA CTT,

SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTTGGAAT GTCACGCATT
GGCATGGAAG TCACACCTT, or

SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTTTA
GTAGTGCTAT CCCCATGTGA TTTTAATAGC TT,

or the complementary sequence thereof,

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCTAAT ATGTTTATCA
CCCGCGAAGA AGCTATTCGT CACGTTTCGTG CGTGGATTGG CTTTGATGT,

SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC
AATCAACACC AATAGTGGTC CAGATGACCA AAT,

SEQ ID 26: CCAAAGTGTG ACTAAGAAAT CTGCTGCTGA GGCATCTAAA
AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC
ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT
AACTAAACAG CACAAGTAGG TTTAGTTAAC TTTAATCTCA CATAGCAATC

TTTAATCAAT GT,
or the complementary sequence thereof.

2. Pair of oligonucleotides, according to claim 1, consisting essentially of:

a first oligonucleotide comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

- SEQ ID 3: TCCACCAGGT GACCAGTTTA AACATCTT,
- 5 SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,
- SEQ ID 5: TACCTCTCCA GCTAGGATTT TCT,
- SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,
- SEQ ID 16: TAGGAACTGG CCCAGAAGCT TCACTT,
- SEQ ID 24: TGCTCCAAGT GCCTCTGCAT TCTT,
- 10 SEQ ID 25: TTGGCATGGA AGTCACACCT T,
- SEQ ID 32: TGCCTATATG GAAGAGCCC,
- SEQ ID 33: TCCCCATGTG ATTTTAATAG CTT,

or the complementary sequence thereof, and

a second oligonucleotide comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

- SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,
- SED ID 7: GAAGCTATTC GTCACGTTTCG,
- SEQ ID 8: TGCGTGGATT GGCTTTGATG T,
- 20 SEQ ID 18: AGGTTTACCC AATAATACTG CGT,
- SEQ ID 19: AGATTCCCTC GAGGCCAGGG CGT,
- SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,
- SEQ ID 27: CCAAAGTGTG ACTAAGAAAT CTGCT,
- SED ID 28: CTCAAGCATT TGGGAGACGT GGT,
- 25 SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,
- SEQ ID 35: TACGATACAT AGTCTACTCT TGT,
- SED ID 36: TAACTAAACA GCACAAGTAG GT,
- SEQ ID 37: TAGCAATCTT TAATCAATGT,

or the complementary sequence thereof.

3. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the replicase gene of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATTT TCTACAGGTG TTAAGTTAGT AGCTGTACCG
ACTGGTTATG TTGACACTGA AAATAACACA GAATTCACCA GAGTTAATGC

5 AAAACCTCCA CCAGGTGACC AGTTTAAACA TCTT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCTAAT ATGTTTATCA

10 CCCGCGAAGA AGCTATTCGT CACGTTCTGT CGTGGATTGG CTTTGATGT, or the complementary sequence thereof.

4. Pair of oligonucleotides, according to claim 3, consisting essentially of:

15 a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 3: TCCACCAGGT GACCAGTTTA AACATCTT,

SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,

20 SEQ ID 5: TACCTCTCCA GCTAGGATTT TCT,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

25 SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,

SEQ ID 7: GAAGCTATTC GTCACGTTCG,

SEQ ID 8: TGCCTGGATT GGCTTTGATG T,

or the complementary sequence thereof.

30 5. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the gene encoding the Nucleocapsid protein of the genome of SARS Coronavirus, said pair consisting of:

35 a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC
AGAAGCTTCA CTT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length
and comprising at least a fragment of 10 nucleotides of:

5 SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC
AATCAACACC AATAGTGGTC CAGATGACCA AAT, or the complementary
sequence thereof.

10 6. Pair of oligonucleotides, according to claim 5,
consisting essentially of:

a first oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
of:

15 SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,
SEQ ID 16: TAGGAACTGG CCCAGAAGCT TCACTT,
or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
20 of:

SEQ ID 18: AGGTTTACCC AATAATACTG CGT,
SEQ ID 19: AGATTCCCTC GAGGCCAGGG CGT,
SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,
or the complementary sequence thereof.

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7. Pair of oligonucleotides, for use as a set in the
amplification of a target sequence located within the gene
encoding the Nucleocapsid protein of the genome of SARS
Coronavirus, said pair consisting of:

30 a first oligonucleotide being 10-50 nucleotides in length
and comprising at least a fragment of 10 nucleotides of:

SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTTGGAAT GTCACGCATT
GGCATGGAAG TCACACCTT, or the complementary sequence thereof,
and

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a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 26: CCAAAGTGTG ACTAAGAAAT CTGCTGCTGA GGCATCTAAA

5 AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC
ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or the complementary sequence thereof.

8. Pair of oligonucleotides, according to claim 7,
10 consisting essentially of:

a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 24: TGCTCCAA GTGCCTCTGC ATTCTT,

15 SEQ ID 25: TTGGCATGGA AGTCACACCT T,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

20 SEQ ID 27: CCAAAGTGTG ACTAAGAAAT CTGCT,

SEQ ID 28: CTCAAGCATT TGGGAGACGT GGT,

SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,

or the complementary sequence thereof.

25 9. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the 3'-Non Coding Region (3'-NCR) of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length
30 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTTTA

GTAGTGCTAT CCCCATGTGA TTTTAATAGC TT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length
35 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT
AACTAAACAG CACAAGTAGG TTTAGTTAAC TTTAATCTCA CATAGCAATC
TTAATCAAT GT, or the complementary sequence thereof.

5 10. Pair of oligonucleotides, according to claim 9,
consisting essentially of:

a first oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
of:

10 SEQ ID 32: TGCCTATATG GAAGAGCCC,
SEQ ID 33: TCCCATGTG ATTTTAATAG CTT,
or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
15 of:

SEQ ID 35: TACGATACAT AGTCTACTCT TGT,
SEQ ID 36: TAACTAAACA GCACAAGTAG GT,
SEQ ID 37: TAGCAATCTT TAATCAATGT,
or the complementary sequence thereof.

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11. Pair of oligonucleotides, according to any of the
claims 1-10, wherein the first oligonucleotide is provided with
a promoter sequence recognized by a DNA dependent RNA
polymerase.

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12. Pair of oligonucleotides, according to claim 11,
wherein the first oligonucleotide consists essentially of the
sequence:

30 SEQ ID 9: aattctaata cgactcacta tagggAAGAT GTTTAACTG
GTCACCTGGT GGA,

SEQ ID 10: aattctaata cgactcacta tagggAACAT AACCAGTCGG
TACAGCTACT A,

SEQ ID 11: aattctaata cgactcacta tagggAGAAA ATCCTAGCTG
GAGAGGTA,

35 SEQ ID 39: aattctaata cgactcacta tagggAGAAG TACCATCTGG GGCTGA,

SEQ ID 40: aattctaata cgactcacta tagggAAGTG AAGCTTCTGG
GCCAGTTCCT A,

SEQ ID 41: aattctaata cgactcacta tagggAAGAA TGCAGAGGCA
CTTGGAGCA,

5 SEQ ID 42: aattctaata cgactcacta tagggAAGGT GTGACTTCCA TGCCAA,
SEQ ID 43: aattctaata cgactcacta tagggGGGCT CTTCCATATA GGCA, or
SEQ ID 44: aattctaata cgactcacta tagggAAGCT ATTAAATCA
CATGGGGA.

10 13. Pair of oligonucleotides, according to any of the
claims 1-12, wherein each oligonucleotide being 15-30
nucleotides in length and comprising at least a fragment of 18
nucleotides, and preferably being 18-26 nucleotides in length
and comprising at least a fragment of 20 nucleotides.

15 14. Oligonucleotide, for use as a probe to detect the
amplified nucleic acid sequence resulting in the amplification
of a target sequence located within the genome of SARS
Coronavirus, said amplification being based on pair of
20 oligonucleotides according to any of claims 1-13, said probe
being 10-50 nucleotides in length and comprising at least a
fragment of 10 nucleotides of:

SEQ ID 12: GTTCGTGCGT GGATTGGCTT TGATGTAGAG GGCTGTCATG
CAACTAGAGA TGCTGT,

25 SEQ ID 21: GGCTACTACC GAAGAGCTAC CCGACGAGTT CGTGGTGGTG
ACGGCAAAAT GAAAGAGCTC AGCCCCAGAT GGTACTTCTA TTACCTAGGA
ACTGGCCCAG AAGCTTCACT TCCCTACGGC GCTAACAAAG AAGGCATCGT
ATGGGTTGCA ACTGAGGGAG CTTGAATAC ACCCAAAGAC CACATTGGCA
CCCGCAATCC TAATAACAAT GCTGCCACCG TGCTACAACT TCCTCAAGGA
30 ACAACATTGC CAAAAGGCTT CTACGCAGAG GGAAGCAGAG GCGGCAGTCA
AGCCTCTTCT CGCTCCTCAT CACGTAGTCG CGGTAATTCA AGAAATTCAA
CTCCTGGCAG CAGTAGGGGA AATTCTCCTG CTCGAATGGC TAGCGGAGGT
GGTGAAACTG CCCTCGCGCT ATTGCTGCTA GACAGATTGA ACCAGCTTGA
GAGCAAAGTT TCTGGTAAAG GCCAACAACA ACAAGGCCAA ACTGTCACTA
35 AGAAATCTGC TGCTGAGGCA TCTAAAAAGC CTCGCCAAAA ACGTACTGCC

ACAAAACAGT ACAACGTCAC TCAAGCATTT GGGAGACGTG GTCCAGAACA
AACCCAAGGA AATTTCGGGG ACCAAGACCT AATCAGACAA,

SEQ ID 38: GCCACCACAT TTTCATCGAG GC,

or the complementary sequence thereof, provided with a
5 detectable label.

15. Oligonucleotide, according to claim 14, wherein the
probe is constituted by a molecular beacon, preferably
consisting of:

10 SEQ ID 13: 5'-[6-FAM]-ccatgggCTGTCATGCAACTAGAGATGCTGTcccattgg-
[DabSyl]-3',

SEQ ID 45: 5'-[6-FAM]-cgcgatGTTTCGTGCGTGGATTGGCTTatcgcg-
[DabCyl]-3',

15 SEQ ID 22: 5'-[6-FAM]-ccatgggCTACTACCGAAGAGCTACCCGACGAcccatgg-
[DabSyl]-3',

SEQ ID 30: 5'-[6-FAM]-ccatggACCAAGACCTAATCAGACAAccatgg-
[DabSyl]-3',

SEQ ID 47: 5'-[6-FAM]-ccatgcGCCACCACATTTTCATCGAgcatgg-[DabSyl]-
3'.

20 16. Use of an oligonucleotides' pair, according to any of
the claims 1-13, in a nucleic acid amplification reaction or as
a probe for the detection of SARS Coronavirus nucleic acid in a
sample.

25 17. Method for the detection of SARS nucleic acid in a
sample wherein the sample is subjected to a nucleic acid
amplification reaction using a pair of oligonucleotides
according to any of the claims 1-13 and suitable amplification
30 reagents and the presence of any amplified nucleic acid is
detected.

35 18. Method according to claim 17, wherein the detection of
any amplified nucleic acid is carried out by reacting the
sample with an oligonucleotide according to claim 14 or 15

under suitable hybridization conditions and detecting the presence of the label in any hybrids formed between the amplified sequence and the probe.

5 19. Method according to claim 17, wherein the amplification technique used is a transcription based amplification technique, preferably the NASBA, and the first oligonucleotide is provided with a promoter sequence recognized by a DNA dependent RNA polymerase.

10 20. Test kit for the detection of SARS Coronavirus in a sample comprising:

 set of oligonucleotides according any of claims 1-13,
 an oligonucleotide comprising a nucleic acid sequence
15 substantially complementary to at least part of the amplified nucleic acid sequence, provided with a detectable label, according to claim 14 or 15, and
 suitable amplification reagents.

20 21. Test kit according to claim 20, wherein suitable amplification reagents enable a transcription based amplification technique, preferably the NASBA.